APPLICATION NOTE

EarlyTox Glutathione Assay Kit on SpectraMax Fluorescence Microplate Readers

Introduction

Apoptosis is a highly regulated cellular program that causes cell death in normal processes such as embryonic development, as well as diseases including cancer and neurodegenerative conditions. A decrease in the level of cellular glutathione (GSH) is commonly observed during the early stages of apoptosis and is triggered by a range of factors including activation of death receptors, stress, and cytotoxic compounds.

The EarlyTox™ Glutathione Assay Kit uses monochlorobimane (MCB), a cell permeant dye with a high affinity for GSH, to detect cellular GSH levels. Reaction of the dye with GSH is catalyzed by endogenous glutathione-S-transferase (GST) enzymes and results in the generation of blue fluorescence with excitation at 394 nm and emission at 490 nm. The fluorescence intensity corresponds to the amount of GSH present in cells, which decreases with apoptosis. Unlike representative competitor assays, the EarlyTox Glutathione Assay enables the use of live, intact cells in a microplate format without the need for cell harvest and centrifugation, lysis, or other time-consuming manipulations that can lead to variability in results.

In this application note we report the use of the EarlyTox Glutathione Assay Kit in combination with SpectraMax® fluorescence microplate readers. Fluorescent signal from MCB can be detected using the microplate reader and rapidly analyzed using a preconfigured protocol in SoftMax® Pro Software (Figure 1).

Materials

- EarlyTox Glutathione Assay Kit
  - Explorer Kit (2-plate size): Molecular Devices P/N R8344
  - Bulk Kit (10-plate size): Molecular Devices P/N R8345
- HeLa cells (ATCC P/N CCL-2)
- Staurosporine (Sigma P/N S5921)
- 96-well black, clear-bottom microplates (Corning P/N 3904)
- SpectraMax fluorescence microplate reader

Benefits

- **Simple workflow—direct measurement in wells with or without medium removal**
- **Increased throughput with microplate format**
- **Preconfigured protocol in SoftMax Pro Software**

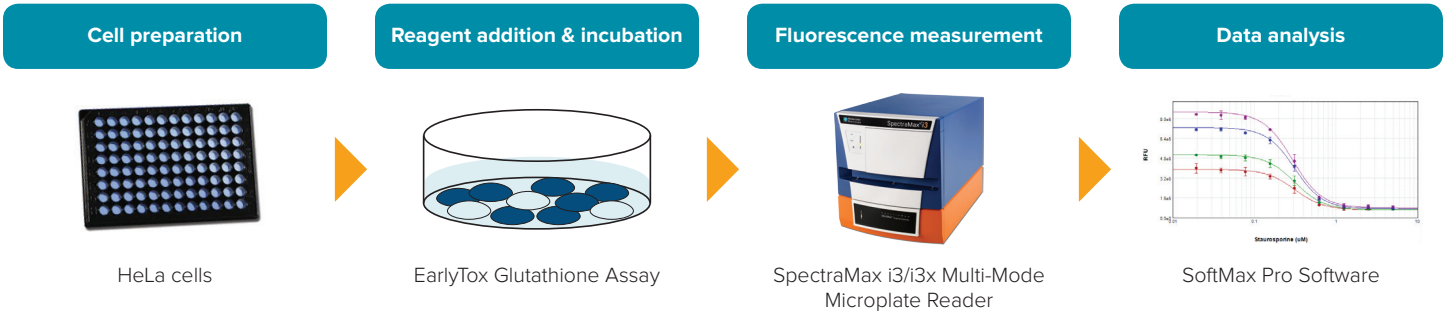

Figure 1: EarlyTox Glutathione Assay workflow.

Methods

HeLa cells were plated at 20,000 cells per well in 100 µL of medium in a 96-well, black, clear-bottom microplate. They were allowed to attach and grow overnight in a 37°C, 5% CO_2 incubator. They were then treated for five hours with a 1:2 dilution series of staurosporine from 5 µM down to 0.02 µM to induce apoptosis.

A 20 µM MCB working solution was prepared by diluting 20 µL of 10 mM MCB stock solution in 10 mL of PBS. Medium was removed from the cells in the assay plate and replaced with 100 µL of MCB working solution. Cells were then incubated at 37°C. After 30 minutes, 1 hour, 2 hours, and 3 hours the plate was taken out of the incubator, and fluorescence intensity was measured in a SpectraMax i3 Multi-Mode Microplate Reader using the settings indicated in Table 1.

Results

HeLa cells treated with staurosporine for five hours exhibited a concentration-dependent decrease in fluorescence as detected with the SpectraMax i3 Multi-Mode Microplate Reader, indicating the decrease of GSH associated with apoptosis. Results were graphed in SoftMax Pro Software using a 4-parameter curve fit. Fluorescence intensity values increased over time, but the same EC_{50} value of 0.3 µM was obtained for assay incubation times ranging from 30 minutes to 3 hours (Figure 2).

Conclusion

The EarlyTox Glutathione Assay Kit, used together with SpectraMax fluorescence microplate readers, provides users a simplified workflow for measuring the decrease in cellular glutathione associated with early stages of apoptosis, with the increased throughput of a microplate format. In contrast to other commercially available glutathione assays, the EarlyTox Glutathione Assay enables users to measure glutathione levels in live, intact cells, within the wells of a microplate, without multiple sample processing steps that can introduce variability.

Parameter	Setting
Read mode	Fluorescence
Read type	Endpoint
Wavelengths	Excitation = 394 nm Emission = 490 nm
PMT and optics	PMT gain: Automatic Flashes per read: 20 Read from bottom*

*Reading from bottom is recommended, but if this feature is not available, top read may be used.

Table 1. Optimized instrument settings for SpectraMax fluorescence microplate readers.

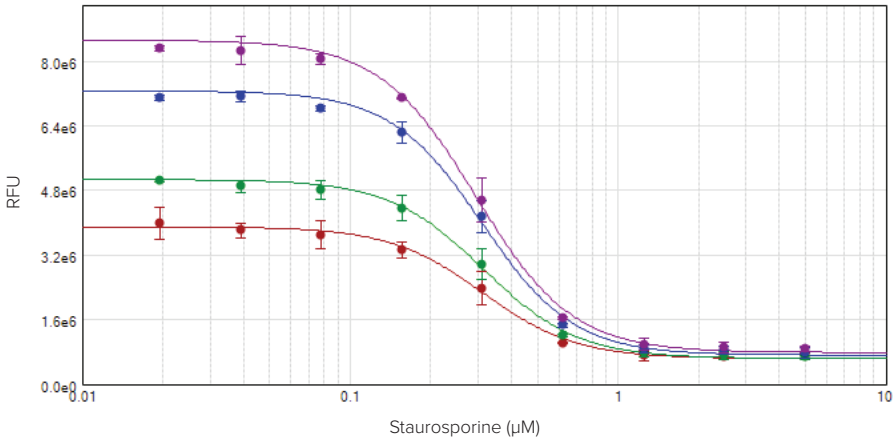

Figure 2: Concentration-response curves for HeLa cells treated with staurosporine. After treatment with staurosporine, cells were incubated in MCB solution for 30 minutes (red), 1 hour (green), 2 hours (blue), or 3 hours (purple). 4-parameter curve fit and data analysis were done in SoftMax Pro Software. EC_{50} values calculated from the curves were 0.3 µM for each time point.

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